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13. ABSTRACT *(Maximum 200)*

We have developed small molecules to alter the transcriptional response of viral genes for the treatment of human breast cancer. We have previously successfully used these compound as therapeutic agents for gene regulation in humans. The transcriptional activity of certain viral genes, including the Epstein-Barr virus (EBV) Thymidine Kinase (TK) gene, is enhanced by these drugs. This work has led to the development of a virus-directed strategy for treating lymphoid malignancies associated with EBV. Exposure to Arginine Butyrate induces the latent TK gene in EBV-infected cells, resulting in susceptibility to ganciclovir. The presence of the Epstein-Barr virus in breast carcinomas of varying histology is strikingly high, raising the possibility of a therapeutic strategy for EBV(+) breast cancers. Our approach initially involves investigation of EBV sequences in breast cancer cell lines and specimens, determination of whether treatment with Arginine Butyrate will induce the viral thymidine kinase gene, and determination *in vitro* of whether the induction of the TK gene and gene product makes the breast cancer cells now susceptible to ganciclovir. The purpose of this proposal is to explore the association of EBV-infection and breast cancer, and to develop and EBV-based strategy for treating breast cancer.

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Progress Report

Douglas V. Faller, Ph.D., M.D.

Virus-Targeted Therapeutic for Breast Cancer

5. Introduction

This work utilizes our development of small molecules (butyrate-related compounds) to alter the transcriptional response of viral genes for the treatment of human breast cancer. We have previously successfully used these compounds as therapeutic agents for gene regulation in humans. The transcriptional activity of certain viral genes, including the Epstein-Barr viral Thymidine Kinase gene, is enhanced by these butyrates. This work has led to the development of a virus-directed strategy for treating lymphoid malignancies associated with the Epstein-Barr virus, including Hodgkin's disease, Burkitt's lymphoma, certain non-Hodgkin's lymphomas, and B cell lymphoproliferative disorders. The Epstein-Barr virus, which is associated with these disorders is not normally susceptible to the nucleoside anti-viral agent, ganciclovir, which requires an active viral Thymidine Kinase for its activity. Exposure to Arginine Butyrate, however, induces the latent Thymidine Kinase gene in Epstein-Barr virus-infected cells, resulting in susceptibility to ganciclovir. We have proposed and successfully carried out (under FDA approval) the treatment of these lymphoid malignancies associated with the Epstein-Barr virus in pilot clinical trials. We are also approved by the FDA to treat EBV(+) solid tumors.

Epstein-Barr virus, in addition to infecting human B cells, can infect epithelial cells. Quite recently, it has been discovered that the Epstein-Barr virus can be found in a wider distribution of lymphoepithelioma-like carcinomas, including the malignant cells of breast cancer. The presence of the Epstein-Barr virus in breast carcinomas of varying histology is strikingly high. EBV is present in 15 of 28 breast cancers. It is most prevalent in medullary carcinomas and is also quite frequent in the (more prevalent) invasive ductal cancers (of 48 invasive ductal carcinomas, 19 were EBV(+)). In addition to EBV genomic DNA detected by PCR, latent membrane viral protein was detected by immunohistology in the epithelial breast tumor cells (but not in surrounding lymphoid cells). No EBV DNA was found using PCR in 12 normal breast specimens. This finding has raised the possibility of a therapeutic strategy for EBV positive breast cancers. Our approach to developing such a therapy would initially involve investigation of EBV sequences in breast cancer cell lines and specimens, determination of whether treatment with Arginine Butyrate will induce the viral thymidine kinase gene, determination *in vitro* of whether the induction of the TK gene and gene product makes the breast cancer cells now susceptible to

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ganciclovir, and enrollment patients with breast carcinoma into the FDA-approved EBV-associated malignancy protocol and determination of their responses.

Recent reports demonstrate that Epstein-Barr Virus (EBV)-infection of the malignant cells can be found in <u>as many as 50% of breast cancers</u>. We have recently developed a virus-directed strategy for treating (EBV)-associated malignancies: Hodgkin's Disease, African Burkitt's lymphoma, certain Non-Hodgkin's lymphomas, and B cell lymphoproliferative disorders (EBV-LPD). This strategy is based on induction of the EBV thymidine kinase (TK) gene in latently-infected tumor cells employing the experimental drug Arginine Butyrate. After induction of the viral TK gene by Arginine Butyrate, addition of the FDA-approved nucleoside anti-viral agent ganciclovir (GCV) then leads to specific killing of virus-infected tumor cells which express the viral TK and spares normal cells. This potential therapy does not depend on the associated EBV genome being causally-related to the breast tumor. Just the presence of the EBV genome in latent form would be predicted to make the breast tumor susceptible to this combination protocol. The purpose of this proposal is to explore the association of EBV-infection and breast cancer, and to develop an EBV-based strategy for treating breast cancer.

6. Body

A. Evaluation of GCV and GCV + Arginine Butyrate on Cancer Cell lines

1. Effects of the butyrates alone *in vitro* on cancer cell lines

We have put Arginine Butyrate and a derivative, monobutyrin, through the NCI tumor screening panel, with the results of that screen appended. Arginine Butyrate and monobutyrin showed significant anti-tumor cell line activity at levels readily and safely reached *in vivo* in humans.

B. Evaluation of Butyrate on <u>Patient-Derived</u> Tumor Specimens

1. Effects of the butyrates alone *in vitro* on tumor specimens
2. Effects of the butyrates plus GCV *in vitro* on tumor specimens

Formal testing of the Butyrates alone, and in combination with GCV on a tumor cell line derived from a patient, who was successfully treated with the combination has been carried out. The combination showed what appeared to be synergistic activity in killing the tumor cell line.

We also demonstrated induction of the EBV-TK by Arginine Butyrate in the patient's tumor cells. To demonstrate induction of the viral Thymidine Kinase gene in the patient's

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tumor cells by Arginine Butyrate, the cell line generated from her was cultured in the presence of different concentrations of Arginine Butyrate for 24 hrs, or treated with 20 ng phorbol myristyl acetate/ml (PMA, a known inducer of EBV-TK and early gene expression), or left untreated. Total cellular RNA was isolated by guanidine thiocyanate/CsCl RNA extraction, quantified, separated by electrophoresis on formaldehyde agarose gels, and transferred to nitrocellulose (Costar, Cambridge, MA). Purified fragments of EBV-TK and β-actin cDNA were radio-labeled with $[\alpha-^{32}P]dCTP$ using random primer synthesis. Hybridizations were carried out at 42°C in 50% formamide for 18 hr. The filters were washed sequentially in 2 x SSC-0.5% SDS and in 0.2 x SSC-0.1% SDS, at room temperature and at 42°C respectively. Autoradiograms were densitometrically scanned for quantitation, using an LKB laser densitometer (Upsalla, Sweden). A ~4 kb RNA transcript, encoding the EBV-TK mRNA, was induced in the Arginine Butyrate-treated tumor cells in a dose-dependent fashion, and in the PMA-treated tumor cells. No transcript was detected in untreated tumor cells, or in Jurkat cells, an EBV(-) lymphoblastoid human T cell line. Induction of EBV-TK mRNA in the patient's tumor cell line was as efficient with Arginine Butyrate at 1 mM as it was with PMA at 20 ng/ml (**Figure 1**). PMA has previously been the most potent known inducer of EBV-TK induction. Steady state plasma levels of butyrate of 0.1-0.7 mM have been reached in humans during infusions of Arginine Butyrate at the rates used for this patient.

Treatment of the established cell line with Arginine Butyrate alone and at concentrations ranging from 1 mM to 500 mM did not inhibit cell line proliferation (**Figure 2**). Similarly, treatment of the cell line with ganciclovir alone at concentrations up to 30 uM did not inhibit proliferation. In contrast, the combination of Arginine Butyrate at 500 mM plus ganciclovir at 10 and 30 uM synergistically inhibited cell proliferation measured by increases in fluorescence (RFU = relative fluorescence units) of the cytoplasmic label calcein (**Figure 2**). Assays of cell viability demonstrated that ganciclovir at 10 uM, 30 uM and 100 uM showed synergy with Arginine Butyrate at 500 mM and 1000 mM in decreasing cell viability at days 3 and 6 as defined by nuclear labeling with membrane impermeant fluorescent dyes (Figure 3). No differences between treatment groups were observed at day 1 (not shown). These effects on tumor cell proliferation and viability were observed at concentrations of Arginine Butyrate and ganciclovir anticipated to be achievable *in vivo*.

C. Investigation of agents which activate the EBV thymidine kinase (TK) in latently-infected cells.

1. Molecular activity of the butyrate compounds

Butyrate is a potent and reversible late G_1 blocker of the cell cycle. Determination of how butyrate blocks the cell cycle at specific points are important for developing this drug as an anti-cancer agent alone and in combination with anti-viral agents. The cell cycle is composed of a series of steps that can be regulated negatively or positively by various factors. A group of related enzymes known as CDKs positively regulate the cell

cycle and are activated by cyclins at specific stages of the cell cycle. Active cyclin-CDK complexes initiate a complex kinase cascade that directs the cell into DNA synthesis and/or mitosis. The cell cycle also involves internal check points that cause arrest when previous events have not been completed as a response to various external agents.

p53 and p21 Control a Major G_1 Checkpoint

Tumor suppressor p53 is involved in a G_1 cell cycle check point. Another check point of p53 in G_2-M has been reported. In normal cells, p53 protein levels are increased as a response to DNA damage, and block the cell cycle at the G_1 check point to ensure the DNA repair either occurs before replication or induces apoptosis to eliminate the DNA defective cells depending upon cycle type. p53 is a DNA binding protein and acts as a transcription factor for a number of genes, including p21.

p21, a CDK inhibitor, is a downstream target and effector of p53 to induce G_1 arrest. p21 mRNA and protein levels are elevated in a p53-dependent manner after DNA damage. Although p21 is induced only in cells having an intact p53 after irradiation, regulation of p21 is independent of p53 after serum stimulation, during cellular differentiation, and after certain drug treatments. In normal proliferating cells with normal p53, p21 is found in a quaternary complex that includes a cyclin, a CDK, and a PCNA. Conversion of active cyclin-CDK complexes into inactive ones is achieved by changing the ratio of p21 to cyclin-CDK, such that active complexes contain a single p21 molecule where as inactive ones include multiple p21 subunits. Conduction of p21 could lead to cell cycle arrest by allowing the amount of cyclin-CDK complexes in the cell to fall below an inhibitory threshold imposed by p21. However, progressive increase of p21 does not result in a precise corresponding loss of kinase activity, but instead causes an abrupt transition from full activity to essentially complete inhibition. More detail studies on the inhibition of CDKs by p21 revealed that p21 associates preferentially with cyclin-CDK complexes rather than with single CDK molecules; as cyclins are synthesized, they bind CDKs and are then inactivated by p21. Therefore, p21 acts as a buffer that incorporates potentially active kinase, and this buffer must be saturated to produce the active kinase needed to catalyze the G_1-S transition. p21 shows selectivity for G_1 and S phase cyclin-CDK complexes and over expression of p21 causes late G_1 arrest of the cell cycle.

Butyrate-dependent induction of nuclear p53 accumulation in cells.

When activated and translocated to the nucleus, p53 induces the expression of genes that mediate withdrawal from the cell cycle (i.e., growth arrest or apoptosis). Since butyrate elicited growth arrest in Swiss 3T3 cells, we tested whether butyrate treatment also induced accumulation of p53 in the nucleus. Quiescent cells were stimulated with PDGF in the absence or presence of butyrate for 12 hours. Nuclei were then isolated and analyzed for p53 content by immunoblotting with anti-p53 antibodies. PDGF treatment alone resulted in some accumulation of p53. This finding has been reported by other

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investigators who have shown that expression of p53 protein (as well as its sub-cellular distribution) is regulated in a cell cycle-dependent manner. However, additional treatment with butyrate resulted in markedly increased levels of nuclear p53 protein. The cytosolic levels of p53 protein were also occasionally slightly elevated following butyrate treatment. However, p53 mRNA levels were not altered in response to butyrate, indicating that the changes in nuclear p53 protein levels that we observed occurred post-transcriptionally.

A causative role of p53 in mediating butyrate-induced cell cycle arrest was investigated using primary cultures of fibroblasts from p53 -/- 'knockout' mice. It was formally possible that levels of expression of dominant-negative p53 were insufficient to sequester all of the endogenous p53, and that sufficient wild type cellular p53 remained in Sc5 cells to form transcriptionally-active complexes and mediate growth arrest in response to butyrate. To independently rule out this possibility, we obtained primary cultures of fibroblasts from transgenic p53 -/- mice (and from wild-type p53+/+ mice as controls), and tested them for susceptibility to butyrate-induced growth arrest. Cultures of p53 +/+ or -/- cells were rendered quiescent by serum-starvation, then stimulated with PDGF in the presence of [³H]-thymidine with, or without butyrate (**Figure 4**). Both lines synthesized DNA in response to PDGF treatment (although the fold stimulation of DNA synthesis in response to PDGF was consistently lower in p53 -/- fibroblasts, due to higher PDGF-independent proliferation). PDGF-stimulated DNA synthesis was inhibited by approximately 50% in p53+/+ cells and by approximately 45% in p53 -/- cells, as a result of butyrate treatment. Therefore, G_1 growth arrest in response to butyrate occurs independently of p53. Yet, butyrate does induce the p53 tumor suppressor protein and mRNA levels (**Figure 5**).

Butyrate-dependent induction of CDK-Inhibitor p21 accumulation in cells.

p53-induced cell cycle arrest is considered to result from the transcriptional induction of a Cyclin Dependent Kinase (CDK)-inhibitor termed variously p21/CIP/WAF (hereafter referred to as p21). We performed RNA blot analysis to test whether butyrate-treatment induced p21 expression. RNA samples from PDGF-stimulated cells treated with or without butyrate were separated on agarose gels and transferred to nitrocellulose. Filters were probed with ³²P-labelled probes for *p21* as well as for the *mdm-2* proto-oncogene, which is also known to be transcriptionally-regulated by p53. *mdm-2* levels were induced by butyrate at time points corresponding to growth arrest and accumulation of nuclear p53 (**Figure 5**). In addition, massive induction in *p21* levels occurred over the same time frame (**Figure 6**). Since p21 is a member of a family of CDK inhibitors that are assumed to have similar functions, we performed northern analysis to test for butyrate-dependent changes in the expression of other known CDK inhibitors. Butyrate did not elicit changes in expression of RNAs corresponding to *p27*, or any other members of the INK family (*p15, p16, p18, p19*) that we examined so far.

These results demonstrate that butyrate can significantly induce p21 expression at

both mRNA and protein levels in the absence of p53 and cause late G_1 arrest. p21 associates preferentially with cyclin-CDK complexes so that the cyclin first binds to CDK and then is inactivated by p21. These observations are consistent with the idea that p21 can function as a titratable buffer and can set the cyclin threshold necessary for cell cycle progression. On the basis of the assumption that two and not more p21 molecules are required for inhibition, a two fold or greater increase in the concentration of p21 should suffice to strongly inhibit cyclin-CD2K kinase activity. The mechanism of action of butyrate and the p53-independent induction pathway of p21 is under investigation.

pRb-deficient cells, however, lack the butyrate-induced checkpoint. In contrast with wild-type 3T3 cells, cultures of fibroblasts from transgenic Retinoblastoma 'knockout' (Rb-/-) mice (provided by Dr. Robert Weinberg) failed to undergo G_1 arrest following butyrate exposure (**Figure 7**). Our finding that pRb-deficient cells lacked the butyrate-dependent checkpoint indicated that pRb may mediate butyrate-induced G_1 arrest. pRb regulates a mid-late G_1 restriction point which is temporally-coincident with the butyrate-induced checkpoint, also suggesting a possible link between pRb and butyrate-induced G_1 arrest. We have determined that butyrate affects the phosphorylation state of endogenous pRb from mitogen-stimulated intact cells (treated with or without butyrate) by immunoblotting with commercially-available antisera against pRb (**Figure 8**).. The effects of butyrate treatment on pRb phosphorylation in intact cells were evident by changes in the relative amounts of slowly-migrating (hyperphosphorylated) and fast-migrating (hypophosphorylated) pRb protein. This preliminary data is consistent with the hypothesis that pRb mediates butyrate-induced G_1 arrest.

We have now shown inhibition of S-phase cyclin expression by butyrate, which is congruent with the effects observed on pRb function. Entry of cells into S-phase requires the prior transduction of certain signaling events. We hypothesize that one (or more) of these important G_1 signaling events is modified as a result of butyrate exposure. Cyclin A is induced by mitogenic stimuli at time points corresponding to the onset of entry into S-phase. Expression of cyclin A is required for growth factor-stimulated entry into S-phase, and requires transduction of prior G_1 signals. Since butyrate inhibits entry into S-phase, we tested whether butyrate would affect mitogen-induced cyclin A expression. In preliminary experiments, butyrate indeed inhibited mitogen-dependent induction of cyclin A protein in a dose-dependent manner (**Figure 9**). Butyrate therefore perturbs an important signaling event which is necessary for S-phase entry. Interestingly, Cyclin A expression is regulated by the E2F family of transcription factors. E2F activity is in turn controlled by pRb. Therefore, the inhibition of cyclin A expression by butyrate provides another potential link between Rb and the butyrate-induced checkpoint.

Butyrate Inhibition of Histone H3 Synthesis

New synthesis of histone H3 is required for transition from late G_1 and entry into S phase, and H3 synthesis and DNA synthesis are tightly coupled. mRNA analysis has

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demonstrated that Arginine Butyrate suppresses histone H3 transcripts markedly and rapidly at concentrations which also inhibit cell proliferation and cause growth arrest (**Figure 10A**). The mechanism by which this occurs is a dramatic decrease in the half-life of H3 histone transcripts. Within 10 min after butyrate treatment, histone H3 mRNA levels have fallen to less than 50% of normal, with decreases to undetectable levels by 30 min (**Figure 10B**). This is in contrast to a half-life of greater than 6 hrs in the absence of butyrate.

2.   Identification of other agents that might activate gene expression

As proposed in the initial application, we have identified two second generation butyrate derivatives, isobutyramide, with 9 ½ hr half-life and which is orally bioavailable, and monobutyrin, which is orally bioavailable and more concentrated than butyrate. Both of these agents induces the EBV TK gene transcriptionally *in vitro*. We have previously put these drugs through the NCI tumor panel, have found GMP sources and manufacture for these drugs, and registered these drugs with the FDA. We have accumulated a great deal of human safety experience with Isobutyramide, and registered this information with the FDA in conjunction with our IND.

D.   Clinical Protocols: GCV + Arginine Butyrate

1.   To study the clinical pharmacology of Arginine Butyrate in combination with Ganciclovir
      a.   plasma half-life
      b.   major routes of elimination

We have collected and are analyzing formal pharmacokinetics on Arginine Butyrate in the new patients treated with the combination protocol, and have registered this data with the FDA in conjunction with our IND.

2.   To assess toxicity or side effects of the therapy
3.   To observe any anti-tumor activity (within the context of a Phase I study)

We have received IND approval for this therapy from the FDA to carry out the clinical study, and amended the protocol to include children from age 5 and above.

We have treated 5 patients with terminal and refractory EBV-associated malignancies to date with the combination of Arginine Butyrate and Ganciclovir. There were minimal side effects in these patients, and dramatic tumor responses in all (**Table I**).

We have treated two patients who withdrew before completing one cycle of therapy. One patient had an EBV(+) nasopharyngeal lymphoma, and withdrew voluntarily from the study. A second patient with a midline lymphoma withdrew after 4 days of treatment.

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Formal case reports on all these patients are being prepared, and are being submitted to the FDA as they are completed.

7. Conclusions

In the first 9 months of research under this grant, we have made good progress towards all of the Specific Aims we have proposed, including the accumulation of data concerning molecular mechanisms, as well as the development of new, orally-bioavailable forms of TK-gene regulating agents. We will continue work as originally outlined and planned.

8.   Appendices

Figures: 1-10

Tables: 1
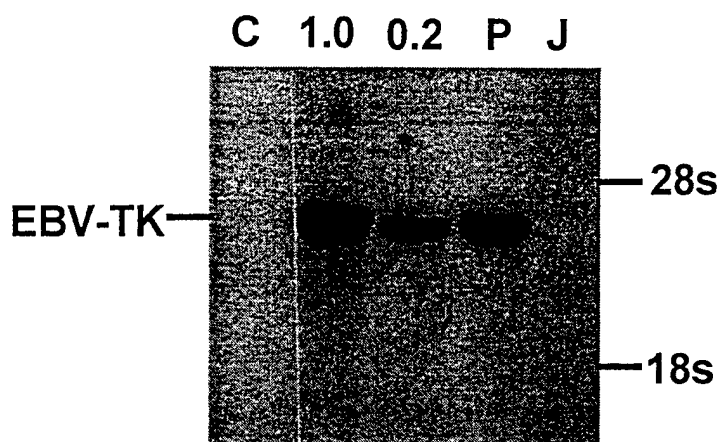
Figure 1. Arginine butyrate induces EBV-TK gene expression in an EBV(+) tumor-derived cell line. Total RNA was extracted from the EBV(+) cell line after incubation for 12 hr in the presence of 0.2 mM (0.2) or 1.0 mM (1.0) arginine butyrate, or 100 nM PMA (P). RNA was also extracted from untreated tumor cells (C), and from the human T cell line Jurkat treated with 100 nM PMA for 12 hr (J). RNA was separated on a formaldehyde-agarose gel, transferred to a nitrocellulose filter, and hybridized with a radiolabeled EBV-TK cDNA probe. The figure is an autoradiogram with the migration positions of 28s and 18s rRNA and of the EBV-TK transcript indicated.
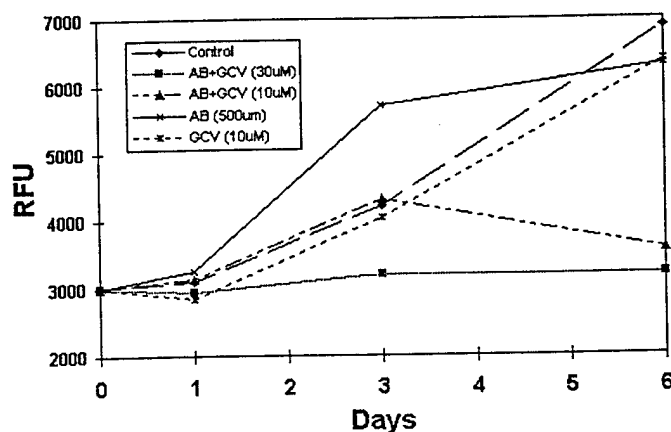
Figure 2. A cytofluorometric microtiter plate assay was used to assess the tumor cell line proliferation during in vitro exposure of the cells to the drugs ganciclovir (GCV) or arginine butyrate (AB), or the combination (AB+GCV). Arginine butyrate at 500 mM plus ganciclovir at 10 and 30 mM inhibited cell proliferation (measured by increases in fluorescence (RFU = relative fluorescent units] of the cytoplasmic label calcein), whereas either drug alone did not.
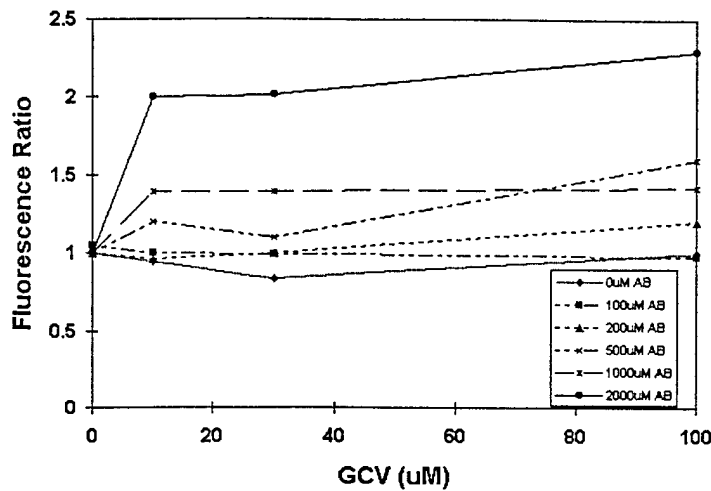
Figure 3. A microfluorometric live/dead assay was used to assess the cytotoxic activity of the established tumor cell line and the drugs ganciclovir (GCV), arginine butyrate (AB) or the combination. A higher fluorescence ratio indicated greater nuclear to cytoplasmic fluorescence and increased cell death. Ganciclovir at 10, 30 and 100 uM showed synergy with arginine butyrate at 500 mM and above when measuring cell viability at day 6.
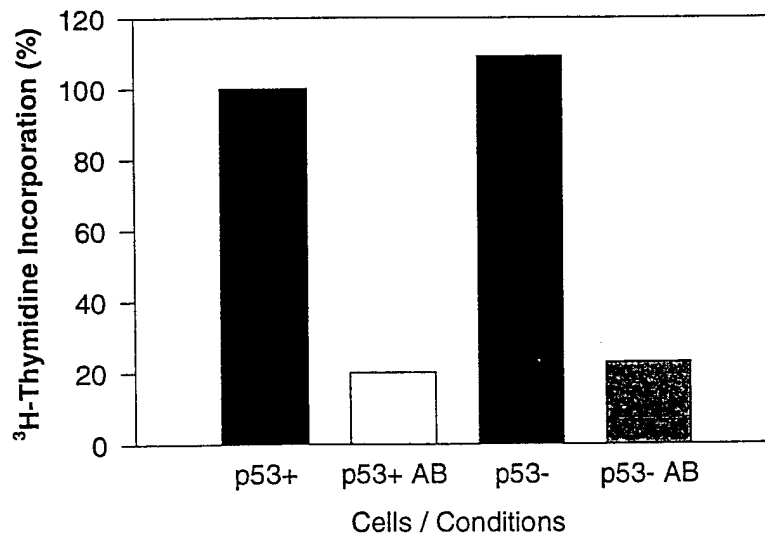
Butyrate Arrest Is Independent of p53

Figure 4: p53 is not required for the butyrate-mediated G_1 checkpoint. Quiescent p53(+/+) or (-/-) MEF cells were left untreated (control) or treated with PDGF, and some plates were also treated with Arginine Butyrate at 2 mM (AB). [³H]-thymidine incorporation was measured at 24 h. Results: Arginine butyrate efficiently blocks cell cycle progression in p53(+) and p53 knockout cells.

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-   0.1   1   2   mM AB

← p53 protein
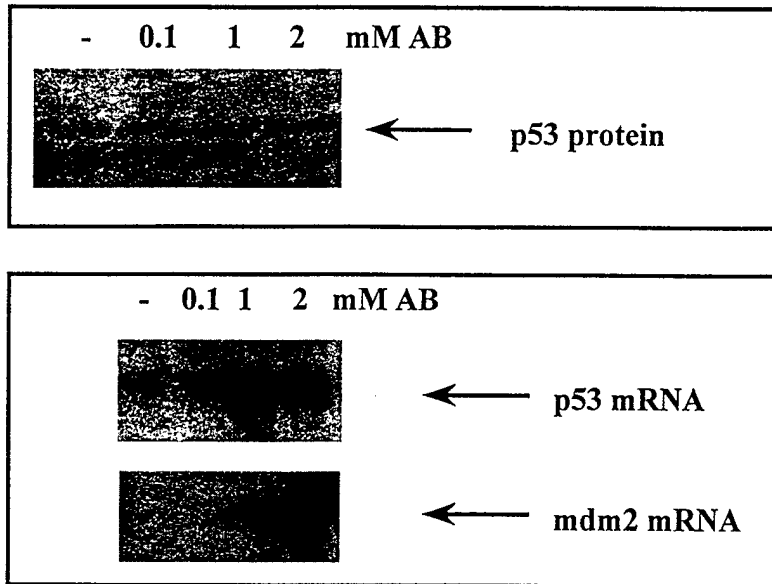
- 0.1 1   2   mM AB

← p53 mRNA

← mdm2 mRNA

Figure 5: p53 mRNA, p53 protein, and p53-Responsive Genes (*mdm2*) are induced by Butyrate. Proliferating fibroblasts were treated with Arginine Butyrate at 0.1, 1, or 2 mM, or left untreated, for 8 hrs. RNA and protein were extracted. Protein samples was analyzed for p53 by immunoblotting with a specific antibody. *p53* and *mdm2* transcripts were detected by hybridization with specific radiolabeled cDNA probes.
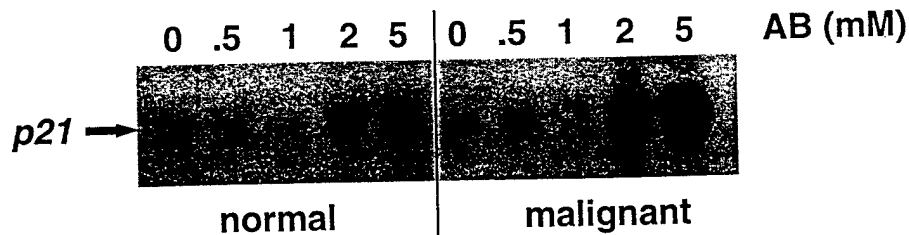
0 .5 1 2 5 | 0 .5 1 2 5   AB (mM)

p21 →

normal | **malignant**

Figure 6: Butyrate treatment induces *p21^{Waf/Cip}* expression. Proliferating normal or Ras-transformed fibroblasts were treated with serum (10%) or left untreated, and some plates were also treated with Arginine Butyrate at 0.1, 1, 2 or 5 mM. RNA was extracted at 4 hrs. *p21^{Waf/Cip}* transcripts were detected by hybridization with a specific radiolabeled cDNA probe. <u>Results:</u> Butyrate treatment induces *p21^{Waf/Cip}* transcripts in normal and malignant cells, at doses which also block cell cycle progression of normal cells and apoptosis of transformed cells.
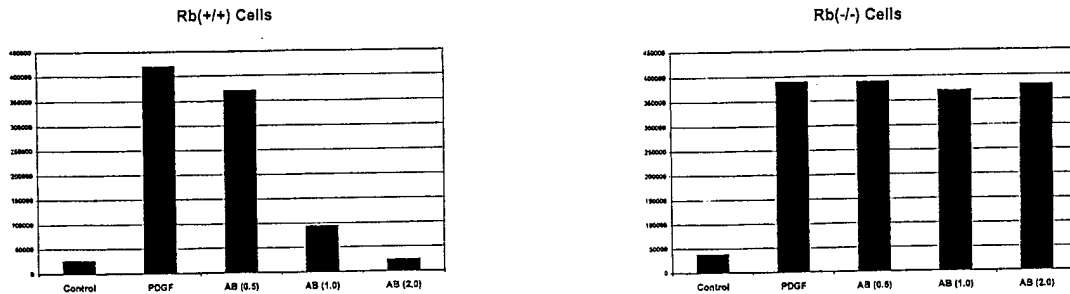
Rb(+/+) Cells   Rb(-/-) Cells

Figure 7: pRb is required for the butyrate-mediated G1 checkpoint. Quiescent pRb(+/+) or (-/-) MEF cells were left untreated (control) or treated with PDGF, and some plates were also treated with Arginine Butyrate at 0.5, 1 and 2 mM. [^{3}H]-thymidine incorporation was measured at 24 h. Results: Arginine butyrate does not block cell cycle progression in pRb knockout cells.

Serum: −   +   +

Butyrate: −   −   +
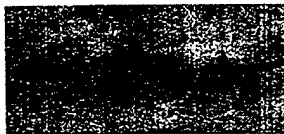
←——— **Rb**

Figure 8: pRb protein hypophosphorylation by mitogenic stimuli is inhibited by Butyrate. Quiescent fibroblasts were treated with serum (10%) or left untreated, and some plates were also treated with Arginine Butyrate at 1 mM. Protein lysates was analyzed for pRb by immunoblotting with a specific antibody. Results: A hypophosphorylated pRb form is induced by mitogenic stimulation, and this is inhibited by butyrate at a dose which also blocks cell cycle progression.

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Butyrate Inhibits Cyclin A Induction

Serum: -   +   +   +   +

mM ArgBut: 0   0   0.5   1   2

← Cyclin A

Figure 9: Butyrate treatment inhibits Cyclin A protein induction. Quiescent fibroblasts were treated with serum (10%) or left untreated, and some plates were also treated with Arginine Butyrate at 0.1, 1, or 2 mM. Protein lysates were made at 12 hrs. Cyclin A protein was detected by immunoblotting with a specific antibody. Results: Serum treatment induces cyclin A protein, and Butyrate inhibits this mitogenic induction of cyclin A at doses which also block cell cycle progression.
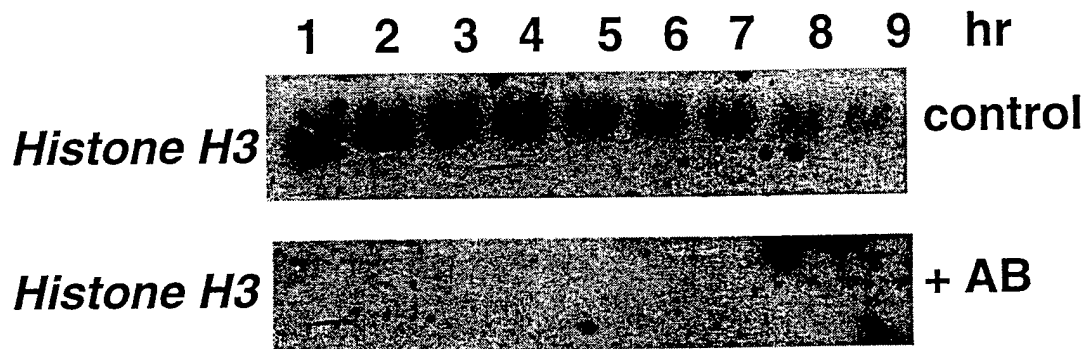
18

1 2 3 4 5 6 7 8 9 hr

Histone H3 — control

Histone H3 — + AB

Figure 10A: Arginine butyrate suppresses histone H3 mRNA levels. Breast cancer cells (MCF-7) were treated with Arginine Butyrate at 1 mM (+AB) or left untreated (control) and total cellular RNA was prepared at 1, 2, 3, 4, 5, 6, 7, 8 and 9 hrs. RNA was separated on a formaldehyde-agarose gel, transferred to a nitrocellulose filter, and hybridized with a radiolabeled Histone H3 cDNA probe. The figure is an autoradiogram with the migration position of the H3 transcript indicated.
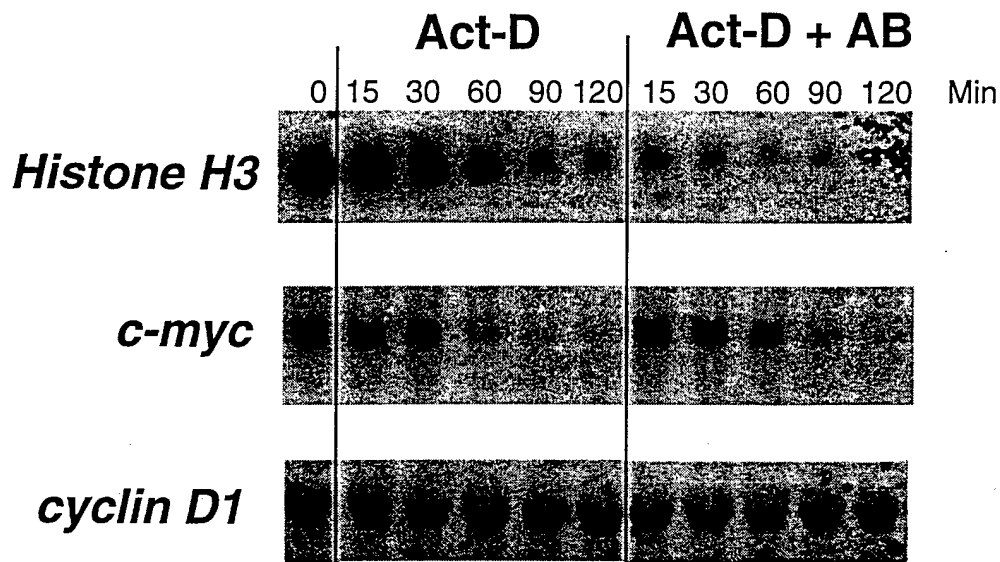
Act-D   **Act-D + AB**

0 | 15 30 60 90 120 | 15 30 60 90 120   Min

Histone H3

c-myc

cyclin D1

Figure 10B: Breast cancer cells (MCF-7) were treated with Arginine Butyrate at 1 mM (+AB) and Actinomycin D (Act-D + AB), or with Actinomycin-D alone (Act-D) and total cellular RNA was prepared pretreatment (0) and at 15, 30, 60, 90, and 120 min. RNA was separated on a formaldehyde-agarose gel, transferred to a nitrocellulose filter, and hybridized with a radiolabeled Histone H3 cDNA probe. The figure is an autoradiogram with the migration position of the H3 transcript indicated.

Table I
Summary of Patients on EBV-Malignancy Protocol

Patient	Age	Prior Hx	Prior Rx	Treatment	Response
1	48	Transplant	Cx, RT, IF	10 d	Complete
2	24	BMT	Cx, RT, IF	10 d	Complete
3	58	Primary	Cx, RT	20 d	Complete
4	3	BMT	no	7 d	Partial
5	30	BMT	Cx, RT	20 d	Complete?

BMT = Bone Marrow Transplant
Cx = Chemotherapy
RT = Radiotherapy
IF = Interferon alpha